The Degradation of Platelet-Activating Factor and Related Lipids: Susceptibility to Phospholipases C and D[1]

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1-*O*-Octadecyl-2-*O*-methyl-*rac*-glycero-3-phosphocholine (ET-18-OCH_3) is an ether-linked lipid that exhibits selective cytotoxicity toward several types of tumor cells and is relatively inactive toward normal cells under the same conditions of treatment. The mechanism of this selective cytotoxicity is unknown. We conducted studies to determine whether this compound is metabolized by phospholipases C and D and, if so, whether sensitive and resistant cells differ in their ability to degrade ET-18-OCH_3 by these enzymes. We have examined the metabolism of the L-isomer of ET-18-OCH_3, 1-*O*-octadecyl-2-*O*-methyl-*sn*-glycero-3-phosphocholine (L-ET-18-OCH_3), by lysophospholipase D of rat liver microsomes and by a phospholipase D from the marine bacterium *Vibrio damsela*. The metabolism of L-ET-18-OCH_3 was also examined in cell culture using Madin-Darby canine kidney cells, human promyelocytic leukemia cells and human myelocytic leukemia cells. In these studies, L-ET-18-OCH_3 and related 1-*O*-alkyl-linked phosphocholine analogs radiolabeled with ^3H in the 1-*O*-alkyl chain were used.

L-ET-18-OCH_3 was not hydrolyzed by lysophospholipase D from rat liver microsomes under conditions where cleavage of 1-*O*-alkyl-2-lyso-*sn*-glycero-3-phosphocholine was observed. However, phospholipase D from the marine bacterium *V. damsela* readily hydrolyzed L-ET-18-OCH_3 to 1-*O*-[^3H]octadecyl-2-*O*-methyl-*sn*-glycero-3-phosphate, demonstrating that L-ET-18-OCH_3 can be degraded by a phospholipase D. Platelet-activating factor (PAF) and lyso-PAF were also substrates for the bacterial phospholipase D.

When intact cells were incubated with radiolabeled L-ET-18-OCH_3, a product was formed that was identified as 1-*O*-[^3H]octadecyl-2-*O*-methyl-*sn*-glycerol. There are two mechanisms that could account for the appearance of this product. The first involves cleavage of the compound by a phospholipase C, resulting in direct release of the diglyceride. The second possible mechanism involves cleavage by a phospholipase D to form the phosphatidic acid analog with subsequent hydrolysis to the diglyceride by a phosphohydrolase. Preliminary data support the phospholipase C–type mechanism. Regardless of which mechanism operates in intact cells, the metabolic degradation of L-ET-18-OCH_3 does not appear to be a significant factor in the selective cytotoxicity of this antitumor agent.

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1-*O*-Octadecyl-2-*O*-methyl-*rac*-glycero-3-phosphocholine (GPC) (ET-18-OCH_3) is an antitumor agent that is selectively cytotoxic to several types of neoplastic cells and is characterized by its relative inactivity toward normal cells (1). Of the three cell lines used in this study, HL60 cells appear to be most sensitive to ET-18-OCH_3, while MDCK and K562 cells are relatively resistant. It has been suggested that the selective cytotoxicity of the drug results from its accumulation in neoplastic cells containing a low activity of the alkyl cleavage enzyme (2–5). According to this hypothesis, normal cells and some types of neoplastic cells were suggested to be resistant to ether lipids due to a higher activity of the alkyl cleavage enzyme, which might prevent accumulation of the alkyl lysophospholipid analogs, thus averting cell death. However, recent studies indicate that the specific activity of the alkyl cleavage enzyme in both sensitive and resistant cells is of the same order of magnitude, suggesting that this enzyme is not responsible for the selectivity of ET-18-OCH_3 (6).

In the present study, we have examined alternative mechanisms of ET-18-OCH_3 hydrolysis that involve phospholipases C and D. Cleavage by a phospholipase C should result in the formation of a diglyceride and a phosphobase. Cleavage by a phospholipase D produces phosphatidic acid and a base. The existence of mammalian phospholipase C that degrades phosphatidylcholine has been demonstrated in Madin-Darby canine kidney (MDCK) cells (7), 3T3-L1 cells (preadipocytic), human promyelocytic leukemia (HL60) cells (8), canine myocardium (9) and HeLa cells (10). A mammalian lysophospholipase D enzyme specific for phosphocholine- and phosphoethanolamine-linked glycerolipids that contain an alkyl linkage at the *sn*-1 position and a free hydroxyl moiety at the *sn*-2 position has also been documented (11–13). We have here examined the possible involvement of phospholipases in the mechanism of action of ET-18-OCH_3. Lysophospholipase D, phospholipase D and phospholipase C were examined for their possible role in ET-18-OCH_3 hydrolysis.

Previous studies on the biological effects of ET-18-OCH_3 have used mixtures of the D- and L-isomers; however, we have prepared the stereochemical homolog of naturally occurring phospholipids, 1-*O*-[^3H]octadecyl-2-*O*-methyl-*sn*-glycero-3-phosphocholine (L-ET-18-OCH_3), for use in studies on ET-18-OCH_3 metabolism.

MATERIALS AND METHODS

Cells. MDCK cells and cell culture reagents were purchased from Flow Laboratories (Rockville, MD). HL60 and human myelocytic leukemia (K562) cells were obtained from American Type Culture Collection (Rockville, MD). Liver microsomes were prepared using a Sprague-Dawley rat.

Reagents. Bovine serum albumin (BSA) and Tris-HCl were obtained from Sigma Chemical Co. (St. Louis, MO). $MgCl_2$ was purchased from Fisher Scientific Co. (Fair

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Abbreviations: GPC, glycero-3-phosphocholine; ET-18-OCH_3, 1-*O*-octadecyl-2-*O*-methyl-*rac*-GPC; PAF, platelet-activating factor, 1-*O*-alkyl-2-acetyl-*sn*-GPC; lyso-PAF, 1-*O*-hexadecyl-*sn*-GPC; TLC, thin layer chromatography; MDCK, Madin-Darby canine kidney; HL60, human promyelocytic leukemia; K562, human myelocytic leukemia; PBS, phosphate-buffered saline; BSA, bovine serum albumin; ID_{50}, the concentration of compound resulting in 50% loss of viability of cells treated 48 hr.

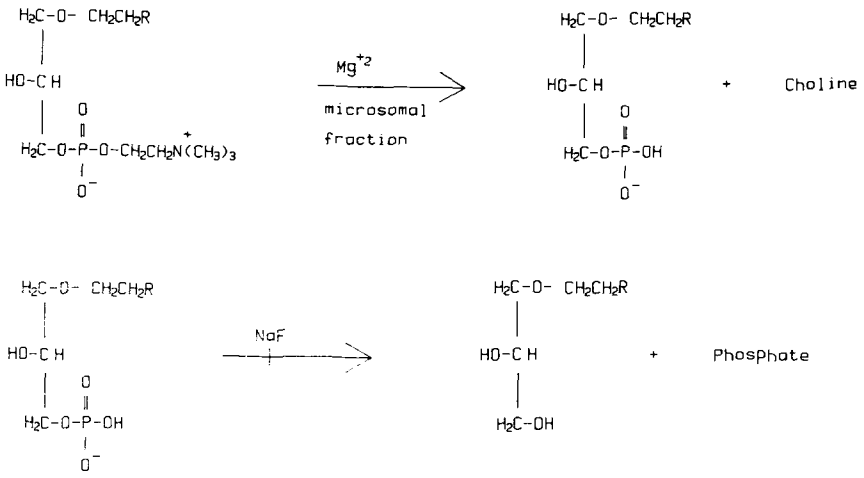

FIG. 1. Lysophospholipase D pathway. In this pathway, the initial product is 1-*O*-alkyl-2-lyso-*sn*-glycero-3-phosphate, which is rapidly converted by a phosphohydrolase in the microsomes to 1-*O*-alkyl-glycerol. The phosphohydrolase can be inhibited by NaF, resulting in the accumulation of 1-*O*-alkyl-*sn*-glycero-3-phosphate. R = $-(CH_2)_{13}CH_3$ or a similar long chain moiety.

Lawn, NJ). Dulbecco's phosphate-buffered saline (PBS) was from Gibco Laboratories (Chagrin Falls, OH), and p-bromophenacyl bromide was from Aldrich Chemical Co. (Milwaukee, WI).

Lipids. 1-*O*-Octadecyl-2-*O*-methyl-*rac*-GPC (ET-18-OCH_3) was a gift from Dr. Wolfgang Berdel, Technical University of Munich (Munich, FRG). 1-*O*-Hexadecyl-*sn*-GPC was purchased from R. Berchtold, Biochemical Laboratory (Bern, Switzerland). 1-*O*-[9,10-^{3}H]Hexadecyl-*sn*-GPC (lyso-PAF) (56 Ci/mmol) and 1-*O*-[9,10-^{3}H]hexadecyl-2-acetyl-*sn*-GPC (PAF) (56 Ci/mmol) were synthesized as described earlier (14). 1-*O*-Hexadecyl-2-*O*-methyl-*sn*-glycerol was a gift from Jeff Surles, University of North Carolina-Chapel Hill, (Chapel Hill, NC). 1-*O*-Octadecenyl-2-*O*-methyl-*sn*-GPC was synthesized by adding a phosphocholine moiety to 1-*O*-octadecenyl-2-*O*-methyl-*sn*-glycerol according to the method of Brockerhoff and Ayengar (16). The synthesis of 1-*O*-octadecenyl-2-*O*-methyl-*sn*-glycerol paralleled the synthesis of 1-*O*-alkyl-2-*O*-ethyl-*sn*-glycerol reported previously (15), substituting selachyl alcohol (1-*O*-octadecenyl-*sn*-glycerol; Western Chemical Industries Ltd., Vancouver, Canada) for chimyl alcohol and methyl methanesulfonate for ethyl methanesulfonate. The 1-*O*-octadecenyl-2-*O*-methyl-*sn*-GPC was tritiated catalytically using palladium (10%) on charcoal to yield 1-*O*-[9,10-^{3}H]octadecyl-2-*O*-methyl-*sn*-GPC (56 Ci/mol).

Lysophospholipase D assay. The lysophospholipase D assays were performed using rat liver microsomes as described by Wykle and Schremmer (11). Protein determinations were made using the method of Bradford (Biorad, Richmond, CA) with BSA as a standard (17). The substrates tested were 1-*O*-[^{3}H]octadecyl-2-*O*-methyl-*sn*-GPC and 1-*O*-[^{3}H]hexadecyl-*sn*-GPC. p-Bromophenacyl bromide, which did not block lysophospholipase D activity, was added to prevent *sn*-2 acylation of the lyso compound (13). The reactions were terminated by lipid extraction using an acidified Bligh and Dyer (18) procedure in which the methanol contained 2% acetic acid. After extraction, the lipids were analyzed by thin layer chromatography (TLC). Following chromatography, the resolved

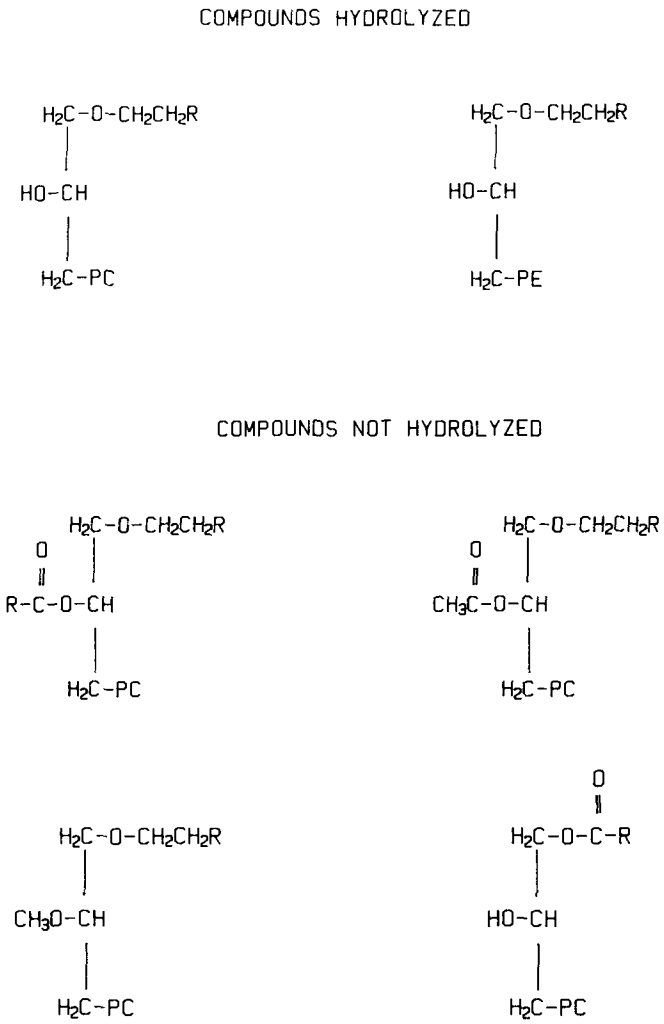

FIG. 2. Substrate specificity of lysophospholipase D. Of the compounds tested, only those containing ether-linked groups at the *sn*-1 position are hydrolyzed. In addition, the 2-hydroxy group must be unesterified. PC, phosphocholine; PE, phosphoethanolamine; R = $-(CH_2)_{13}CH_3$ or a similar long chain moiety.

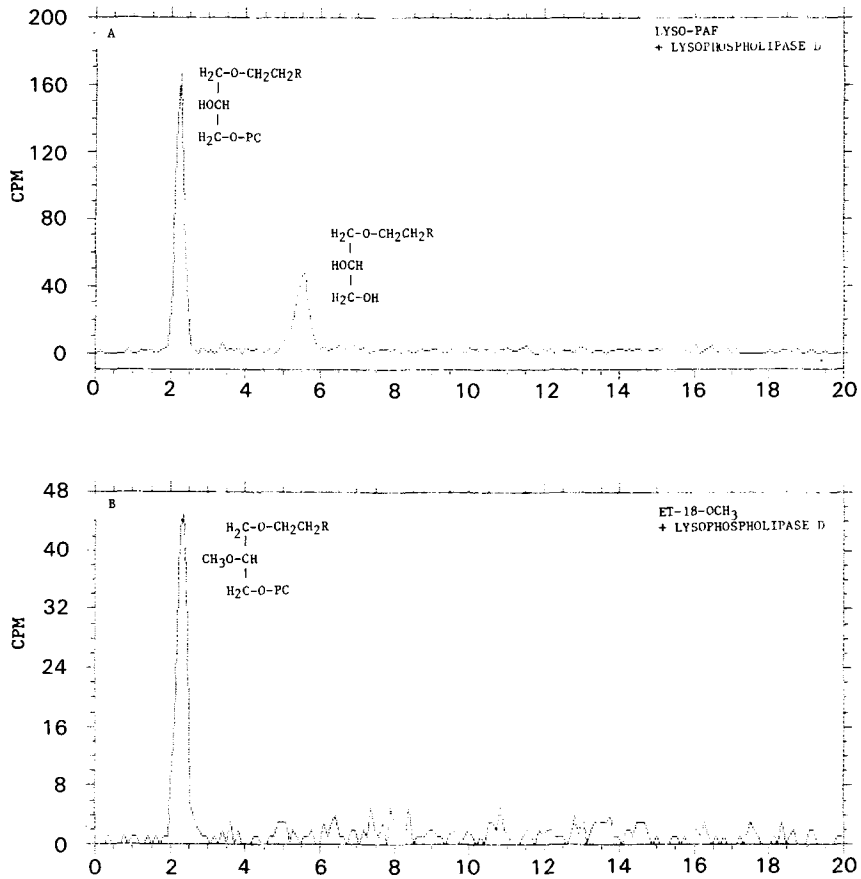

FIG. 3. Relative susceptibility of 1-*O*-[^{3}H]alkyl-2-lyso-*sn*-GPC (lyso-PAF) and 1-*O*-[^{3}H]alkyl-2-*O*-methyl-*sn*-GPC (L-ET-18-OCH_3) to rat liver lysophospholipase D. Lyso-PAF was hydrolyzed by the enzyme under the experimental conditions; L-ET-18-OCH_3 was not. Each 3-ml incubation mixture contained $MgCl_2$ (5 mM), Tris-HCl buffer (0.1 M, pH 7.1), rat liver microsomes (0.5 mg protein), p-bromophenacyl bromide (0.13 M) added directly in 20 μl acetone, and a combination of the respective unlabeled substrates (17 nmol) and radiolabeled substrates (300,000 dpm; 2 pmol) added in 20 μl ethanol. Each incubation was shaken at 37 C for 10 min, and the reactions were terminated by extraction. The lipids were then dried under N_2, resuspended in chloroform/methanol (9:1, v/v) and separated on layers of Silica Gel 60 by developing in ethyl ether/water (100:0.5, v/v) or in chloroform/methanol/glacial acetic acid/water (50:25:8:3, v/v/v/v). The samples depicted in this figure were chromatographed in the solvent system ethyl ether/water (100:0.5, v/v). The major peaks are identified by the adjacent structures, based on migration with standards. In Figures 3–5, radioactivity was measured using a Bioscan radiochromatogram imaging system and is expressed as counts per minute. The abscissa represents distance in cm on the thin layer chromatography plate. Scans of the entire sample lane are shown. Origin, 1–2 cm; solvent front, 18–20 cm.

lipids were visualized by exposing the plates to iodine vapors; radiolabeled products were located using a radiochromatogram imaging system (Bioscan Inc., Washington, DC) (Fig. 3). Regions containing radiolabeled lipid were then scraped and counted using a Packard liquid scintillation counter. Further details of the experimental procedures are given in the figure legends.

Bacterial phospholipase D assay. The phospholipase D enzyme isolated from the marine bacterium *Vibrio damsela* was a gift from A. S. Kreger (Bowman Gray School of Medicine, Winston-Salem, NC). This enzyme preparation was from the stage-4 purification pool with a specific activity of 2×10^6 hemolytic units/mg protein and a concentration of 1.47 mg protein/ml (19). Details of incubations are given in the legends of Figures 4 and 5. Substrates tested were 1-*O*-[^{3}H]octadecyl-2-*O*-methyl-*sn*-GPC (1.5×10^6 dpm, 12 pmol), 1-*O*-[^{3}H]hexadecyl-*sn*-GPC (6.2×10^5 dpm, 5 pmol), 1-*O*-[^{3}H]hexadecyl-2-acyl-*sn*-GPC (9×10^5 dpm, 7 pmol) and 1-*O*-[^{3}H]hexadecyl-2-acetyl-*sn*-GPC (1.0×10^6 dpm, 8 pmol).

Incubations of ET-18-OCH_3 with intact cells. MDCK cells were cultured as a monolayer in Dulbecco's modification of Eagle's medium supplemented with 10% (v/v) fetal bovine serum, 100 units of penicillin/ml, 100 μg of streptomycin/ml, 0.22% Na_2HCO_3 and 2 mM L-glutamine. HL60 and K562 cells were cultured in suspension in RPMI 1640 medium with the same supplements. For these experiments, the cells were pelleted, resuspended in medium containing radiolabeled L-ET-18-OCH_3 and cultured in 35-mm petri dishes during the 48-hr time course (Fig. 6). The concentration of L-ET-18-OCH_3 (24 nM) in these experiments was 100-fold less than the

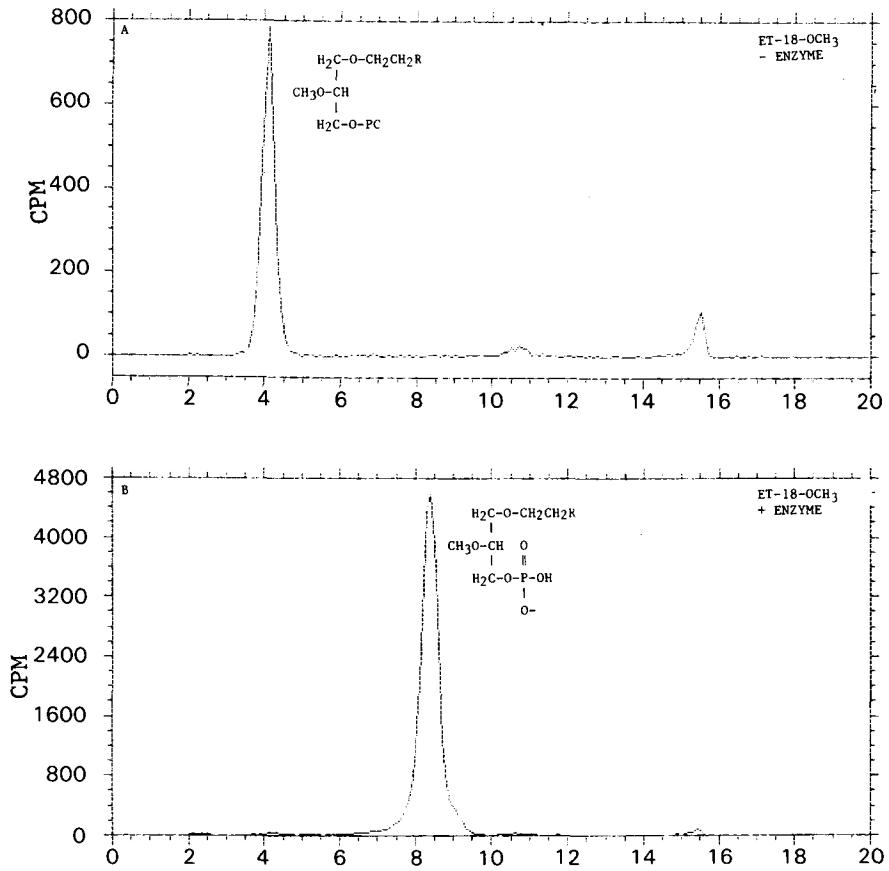

FIG. 4. Hydrolysis of 1-*O*-[^{3}H]octadecyl-2-*O*-methyl-*sn*-GPC to 1-*O*-[^{3}H]octadecyl-2-*O*-methyl-*sn*-glycero-3-phosphate by phospholipase D isolated from the bacterium *Vibrio damsela*. Each incubation mixture in a final volume of 1 ml phosphate-buffered saline was shaken for 30 min at 37 C and contained 1-*O*-[^{3}H]octadecyl-2-*O*-methyl-*sn*-GPC (1.5 × 10^6 dpm, 12 pmol) dried under N_2 and sonicated in 400 μl PBS and (A) no enzyme or (B) 29.4 μg enzyme. The reactions were terminated by lipid extraction as described in Materials and Methods for lysophospholipase D assays. Samples were analyzed by thin layer chromatography using the solvent system chloroform/methanol/glacial acetic acid/water (50:25:8:3, v/v/v/v).

ID_{50} (the concentration of compound resulting in 50% loss of viability of cells treated 48 hr) of the sensitive HL60 cell line. After each incubation period, the cells and fluids (medium) were extracted separately using an acidified Bligh and Dyer (18) procedure in which the methanol contained 2% acetic acid. Lipids were analyzed by TLC using solvent systems described below.

RESULTS AND DISCUSSION

Hydrolysis of L-ET-18-OCH$_3$ by lysophospholipase D. Lysophospholipase D has been shown to hydrolyze 1-*O*-alkyl-linked choline- or ethanolamine-containing phosphoglycerides but not the corresponding 1-*O*-acyl compounds (13) (Figs. 1 and 2). The enzyme is found in a number of tissues, requires Mg^{+2} for activity and does not act on substrates esterified at the *sn*-2 position (11,12). The cellular role of this novel enzyme is unknown. In the present study, when radiolabeled 1-*O*-hexadecyl-*sn*-GPC (lyso-PAF) was incubated with rat liver microsomes in the presence of Mg^{+2}, the substrate was hydrolyzed to 1-*O*-hexadecyl-*sn*-glycerol (Fig. 3A) as reported by Wykle and Schremmer (11). It was also reported that PAF could be cleaved by lysophospholipase D if the acetate group was first removed by an acyl hydrolase present in the microsomes to form the lyso compound (13).

To establish that the enzyme under study was the Mg^{+2} requiring lysophospholipase D described by Wykle and Schremmer (11), the ion requirements of the enzyme were investigated. When the microsomal enzyme preparation was treated with 10 mM EDTA without the addition of Mg^{+2}, 1-*O*-hexadecyl-*sn*-GPC was not hydrolyzed to 1-*O*-hexadecyl-*sn*-glycerol (data not shown). This confirmed that the enzyme being assayed was the lysophospholipase D described earlier.

We tested whether L-ET-18-OCH$_3$ could be hydrolyzed directly by lysophospholipase D. When radiolabeled L-ET-18-OCH$_3$ was incubated with rat liver microsomes in the presence of Mg^{+2}, hydrolysis did not occur (Fig. 3B). These findings are in agreement with earlier observations that a free hydroxy group is required at the *sn*-2 position (11–13). Unless a mechanism is available to remove the 2-*O*-methyl group, lysophospholipase D does not appear to play a role in the metabolism of L-ET-18-OCH$_3$.

Hydrolysis of L-ET-18-OCH$_3$ by a bacterial phospholipase D. Several phosphocholine-containing lipids were

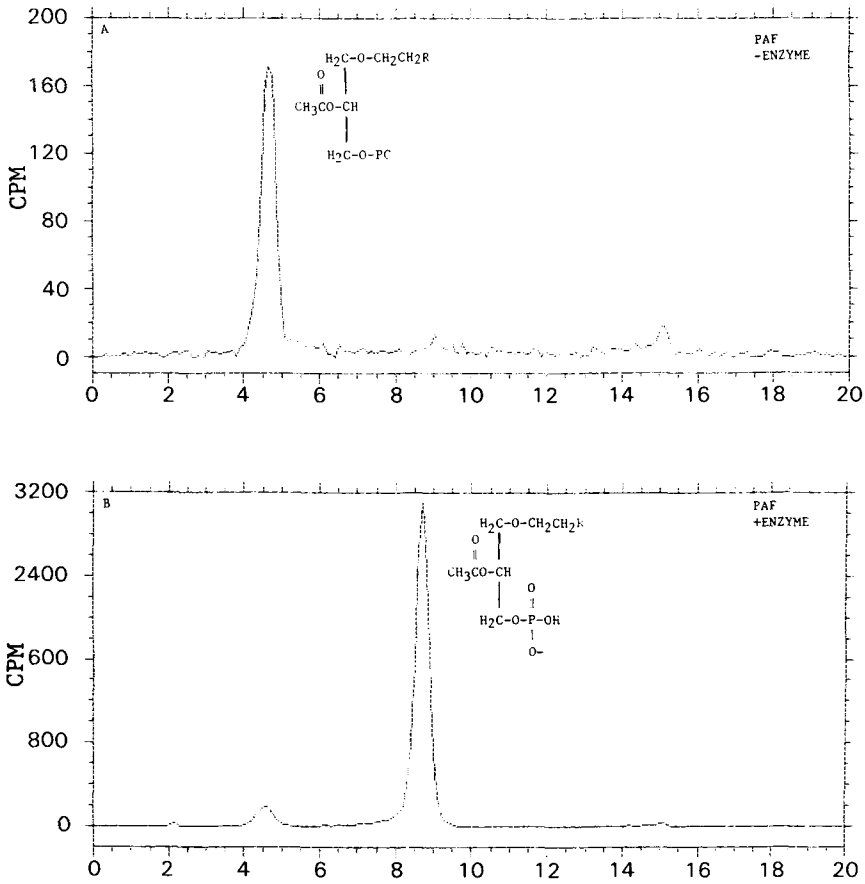

FIG. 5. Hydrolysis of 1-*O*-[^{3}H]hexadecyl-2-*O*-acetyl-*sn*-GPC (platelet-activating factor) to 1-*O*-[^{3}H]hexadecyl-2-*O*-acetyl-*sn*-glycero-3-phosphate by bacterial phospholipase D. The incubation mixture in a final volume of 1 ml contained 1-*O*-[^{3}H]hexadecyl-2-acetyl-*sn*-GPC (1.0×10^6 dpm, 8 pmol) added as described in Figure 4. Incubation times and other conditions as well as methods for analysis of the products were the same as described in Figure 4.

tested as substrates for phospholipase D isolated from the bacterium *Vibrio damsela* (19). Figure 4 shows the degradation of L-ET-18-OCH_3 to 1-*O*-[^{3}H]octadecyl-2-*O*-methyl-*sn*-glycero-3-phosphate by this enzyme. This demonstrated that L-ET-18-OCH_3 is susceptible to metabolism by a phospholipase D and provided a standard for the reaction product of phospholipase D cleavage. Figure 5 shows the degradation of 1-*O*-[^{3}H]hexadecyl-2-acetyl-*sn*-GPC (PAF) to 1-*O*-[^{3}H]hexadecyl-2-acetyl-*sn*-glycero-3-phosphate. Other substrates tested were 1-*O*-[^{3}H]hexadecyl-2-acyl-*sn*-GPC and 1-*O*-[^{3}H]hexadecyl-*sn*-GPC (lyso-PAF). These compounds were also hydrolyzed to the corresponding phosphatidic acid by the bacterial phospholipase D (data not shown).

Hydrolysis of L-ET-18-OCH_3 in intact cells. Figure 6 shows the loss of radiolabeled L-ET-18-OCH_3 from the medium and uptake by MDCK, HL60 and K562 cells. The data points represent relative amounts of label since conditions for recovery were not optimal in these studies. Loss of label due to desaturation at position 9,10 cannot be ruled out. We have not explored this possibility. We have ascribed the loss of label to adsorption to the petri dishes due to the high radiospecific activity of the [^{3}H]L-ET-18-OCH_3 employed (56 Ci/mmol); similar low recoveries are observed in studies using ^{3}H PAF of high radiospecific activity. The concentration of [^{3}H]L-ET-18-OCH_3 used in these studies was 24 nM, whereas the ID_{50} has been reported as 2.5 μM (20). Figure 7 shows the appearance of the hydrolysis product from each cell line. This product, which appeared in both cell and fluid extracts, was identified by TLC as the diglyceride 1-*O*-[^{3}H]octadecyl-2-*O*-methyl-*sn*-glycerol. In two neutral lipid TLC systems, hexane/ethyl ether/formic acid (90:60:4, v/v/v) and ethyl ether/water (100:0.5, v/v), the hydrolysis product migrated (Rf = 0.5 in the ethyl ether/water [100:0.5, v/v] solvent system) with authentic 1-*O*-hexadecyl-2-*O*-methyl-*sn*-glycerol. A TLC zonal scan of products isolated from HL60 fluids after incubation with [^{3}H]ET-18-OCH_3 for 48 hr showed the radiolabeled hydrolysis product in zones 16–21 (Fig. 8). The 1-*O*-hexadecyl-2-*O*-methyl-*sn*-glycerol standard was visualized by iodine staining or by charring the standard lanes. The Rf value of the hydrolysis product in the ethyl ether/water (100:0.5, v/v) solvent system increased after acetylation to 1-*O*-alkyl-2-*O*-methyl-3-acetylglycerol (data not shown). This demonstrated the presence of a free hydroxyl moiety in the radiolabeled hydrolysis product.

No evidence for 1-*O*-alkyl cleavage enzyme products was obtained. Alkyl cleavage activity should have resulted in the appearance of labeled fatty aldehyde and

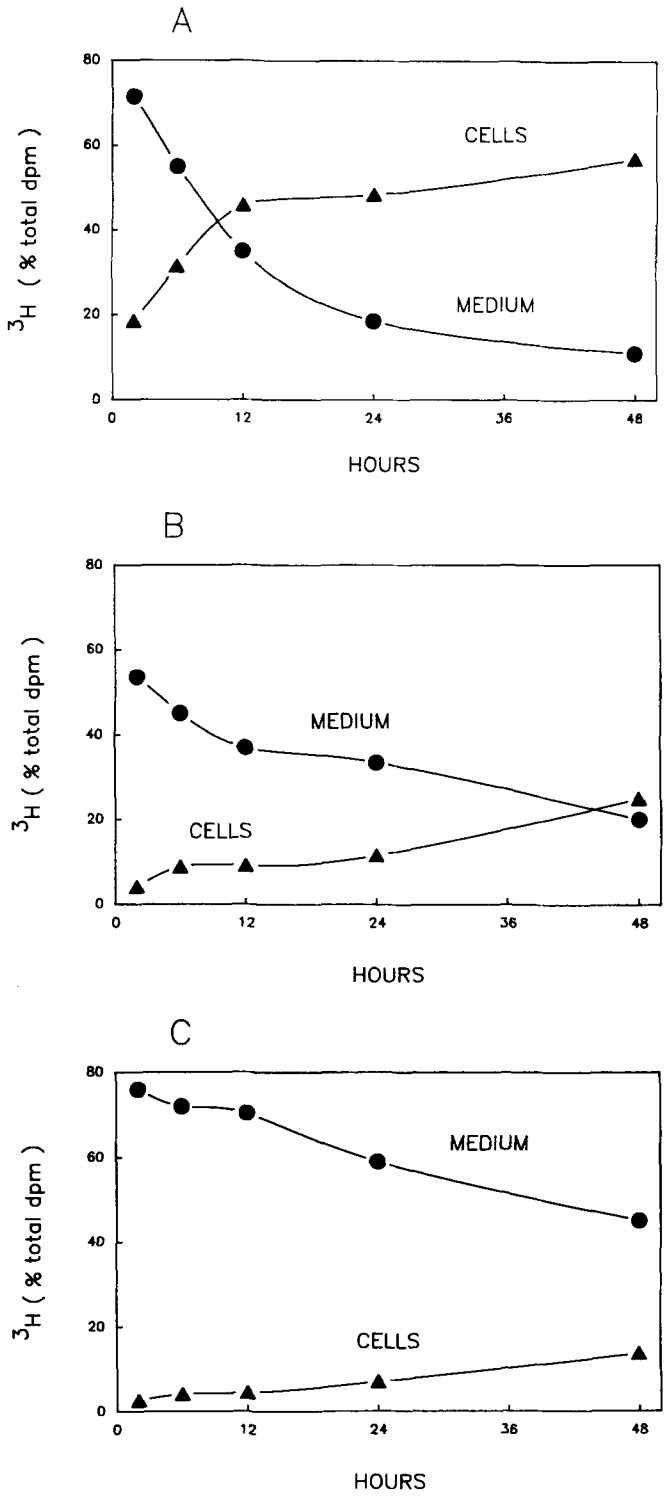

FIG. 6. Loss of [^{3}H]L-ET-OCH$_3$ from the medium and uptake by (A) MDCK, (B) HL60 and (C) K562 cells. For these studies, the cells were pelleted, resuspended in 1 ml of medium containing [^{3}H]L-ET-18-OCH$_3$ (24 nM, 3 × 10^6 dpm) and incubated in 35-mm petri dishes for the various times shown. Approximately 6 × 10^5 cells per sample were used. After each incubation period, the cells and fluids (medium) were extracted and analyzed by thin layer chromatography. The neutral lipid solvent systems used were hexane/ethyl ether/formic acid (90:60:4, v/v/v) or ethyl ether/water (100:0.5, v/v). The basic phospholipid solvent system used was chloroform/methanol/ammonium hydroxide (65:35:8, v/v/v). Values are percentage of total [^{3}H]ET-18-OCH$_3$ (dpm) added to each sample that was recovered in the cell extract (▲) or fluid extract (●).

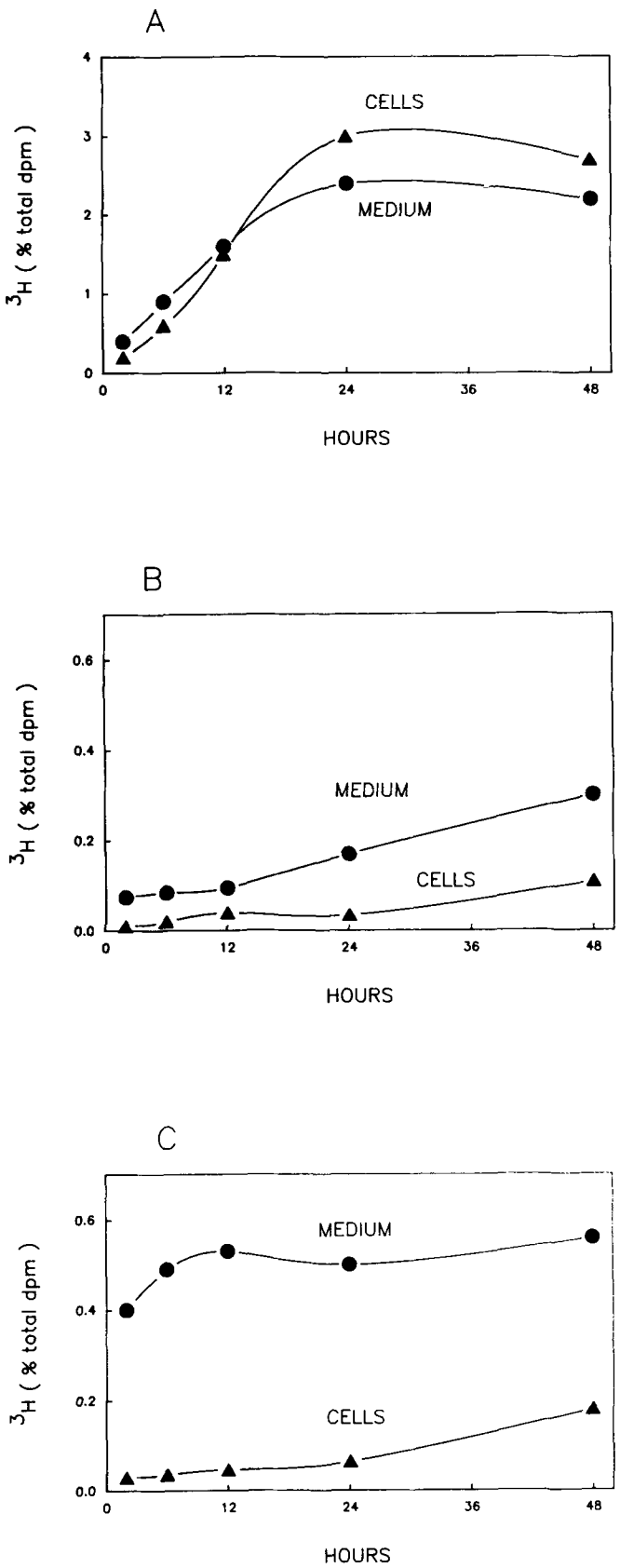

FIG. 7. The formation of 1-*O*-[^{3}H]alkyl-2-*O*-methylglycerol by (A) MDCK, (B) HL60 and (C) K562 cells after incubation with [^{3}H]L-ET-18-OCH$_3$. These are the same samples as in Figure 6. Values are percentage of total dpm added to the sample that were recovered as radiolabeled diglyceride in the cell extract (▲) or fluid extract (●).

fatty acid, which would be expected to incorporate into various cellular lipids. Hoffman and Snyder (6) recently compared alkyl cleavage enzyme activity in cells that demonstrate different sensitivities to ET-18-OCH_3. In contrast to previous studies by other investigators, they found that microsomal preparations from sensitive (HL60) and resistant (K562, MDCK) cells did not differ significantly in alkyl cleavage enzyme activity. They also reported the appearance of the metabolic products 1-*O*-alkyl-2-methoxyglycerol and 1-*O*-alkylglycerol after incubation of HL60, K562 and MDCK cells for 24 hr with 0.5 μM ET-18-OCH_3.

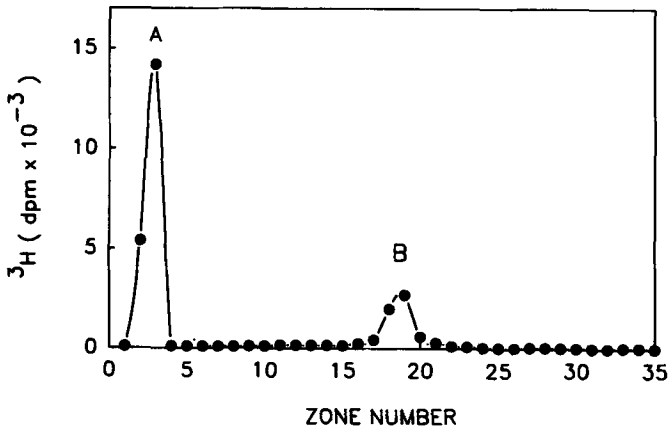

FIG. 8. Zonal scan of a thin layer chromatogram showing the migration of [^{3}H]L-ET-18-OCH_3 and the radiolabeled hydrolysis product, both isolated from HL60 fluids after incubation with [^{3}H]L-ET-18-OCH_3 for 48 hr. The labeled product at zones 16–21 migrated with authentic 1-*O*-hexadecyl-2-*O*-methyl-*sn*-glycerol standard. Each zone division represents a 5-mm region of the thin layer chromatographic sample lane. The origin was at zone 3; the solvent front corresponded to zone 35. (A) [^{3}H]L-ET-18-OCH_3; (B) diglyceride (1-*O*-hexadecyl-2-*O*-methyl-*sn*-glycerol).

The appearance of a radiolabeled diglyceride metabolite suggested a phospholipase C-type mechanism of hydrolysis of ET-18-OCH_3 in whole cells. However, it was also important to consider an alternative mechanism in which a phospholipase D enzyme coupled with a phosphohydrolase could result in the appearance of a diglyceride product. The second mechanism is analogous to that of lysophospholipase D in rat liver microsomes mentioned above. Figure 9 shows the reaction scheme for each of the possible mechanisms of ET-18-OCH_3 hydrolysis.

A basic phospholipid TLC solvent system (chloroform/methanol/ammonium hydroxide [65:35:8, v/v/v]) capable of resolving phosphatidic acid from other components was used to determine if the metabolite 1-*O*-[^{3}H]octadecyl-2-*O*-methyl-*sn*-glycero-3-phosphate was present; none was detected. This evidence supports a phospholipase C-type mechanism of hydrolysis (Fig. 9A), but further experiments must be conducted to clarify this mechanism.

These studies demonstrate that ET-18-OCH_3 is very poorly metabolized by all the cell lines tested. In addition, the liver lysophospholipase does not act on the compound. Based on earlier findings and those reported here, ET-18-OCH_3 is not a substrate for the alkyl cleavage enzyme. The pattern of uptake of ET-18-OCH_3 and release

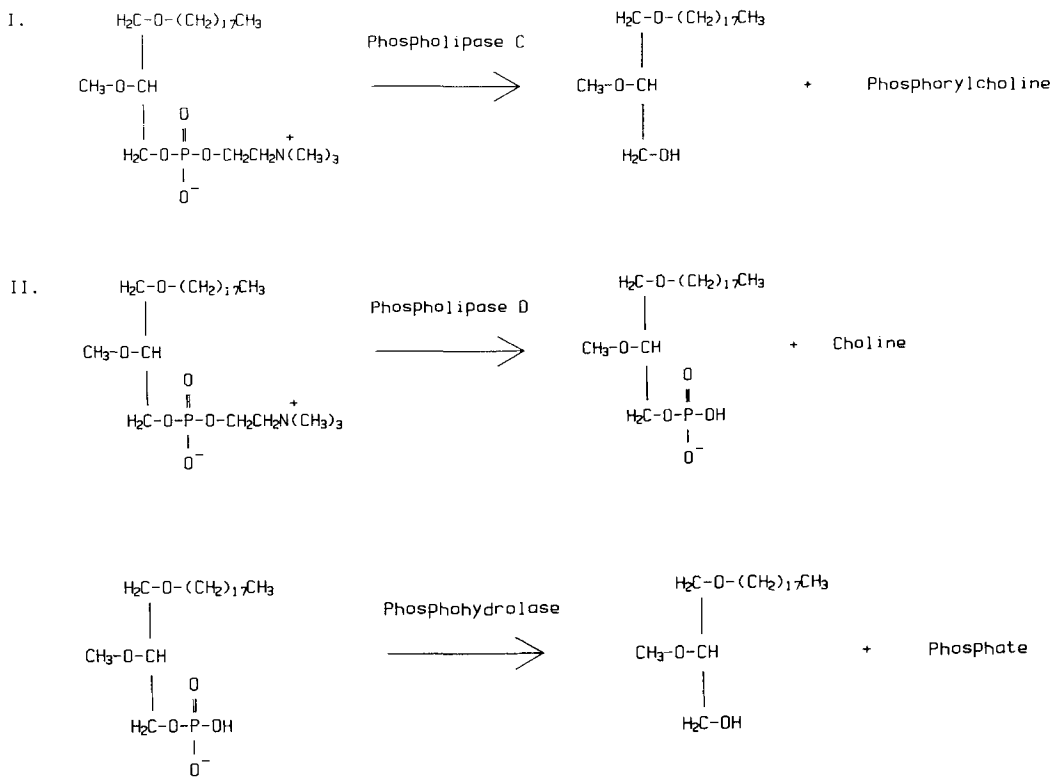

FIG. 9. Two possible pathways explaining the appearance of a radiolabeled diglyceride after incubation of cells with [^{3}H]L-ET-18-OCH_3.

of diglyceride product varied among the cell lines tested. However, these data did not suggest a correlation between cytotoxicity of ET-18-OCH_3 and metabolic degradation of this compound. The selective cytotoxicity of ET-18-OCH_3 toward tumor cells does not appear to be attributable to differences in the catabolism of the compound in normal versus neoplastic cells.

ACKNOWLEDGMENTS

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REFERENCES

1. Berdel, W.E., Andreesen, R., and Munder, P.G. (1985) in *Phospholipids and Cellular Regulation* (Kuo, J.F., ed.) Vol. 2, pp. 41-73, CRC Press, Boca Raton, FL.
2. Soodsma, J.F., Piantadosi, C., and Snyder, F. (1970) *Cancer Res. 30,* 309-311.
3. Andreesen, R., Modolell, M., Weltzien, H.U., Eibl, H., Common, H.H., Lohr, G.W., and Munder, P.G. (1978) *Cancer Res. 38,* 3894-3899.
4. Berdel, W.E., Greiner, E., Fink, U., Stavrou, D., Reichert, A., Rastetter, J., Hoffman, D.R., and Snyder, F. (1983) *Cancer Res. 43,* 541-545.
5. Andreesen, R., Osterholz, J., Luckenbach, G.A., Costabel, U., Schulz, A., Speth, V., Munder, P.G., and Lohr, G.W. (1984) *J. Natl. Cancer Inst. 72,* 53-59.
6. Hoffman, D.R., Hoffman, L.H., and Snyder, F. (1986) *Cancer Res. 46,* 5803-5809.
7. Daniel, L.W., Waite, M., and Wykle, R.L. (1986) *J. Biol. Chem. 261,* 9128-9132.
8. Besterman, J.M., Duronio, V., and Cuatrecasas, P. (1986) *Proc. Natl. Acad. Sci. USA 83,* 6785-6789.
9. Wolf, R.A., and Gross, R.W. (1985) *J. Biol. Chem. 260,* 7295-7303.
10. Guy, G.R., and Murray, A.W. (1982) *Cancer Res. 42,* 1980-1985.
11. Wykle, R.L., and Schremmer, J.M. (1974) *J. Biol. Chem. 249,* 1742-1746.
12. Wykle, R.L., Kraemer, W.F., and Schremmer, J.M. (1977) *Arch. Biochem. Biophys. 184,* 149-155.
13. Wykle, R.L., Kraemer, W.F., and Schremmer, J.M. (1980) *Biochim. Biophys. Acta 619,* 58-67.
14. Wyrick, S.D., McClanahan, J.S., Wykle, R.L., and O'Flaherty, J.T. (1985) *J. Lab. Compd. Radiopharm. 22,* 1169-1174.
15. O'Flaherty, J.T., Wykle, R.L., Miller, C.H., Lewis, J.C., Waite, M., Bass, D.A., McCall, C.E., and DeChatelet, L.R. (1981) *Am. J. Pathol. 103,* 70-78.
16. Brockerhoff, H., and Ayengar, N.K.N. (1978) *Lipids 14,* 88-89.
17. Bradford, M.M. (1976) *Anal. Biochem. 72,* 248-254.
18. Bligh, E.G., and Dyer, W.J. (1959) *Can. J. Biochem. Physiol. 37,* 911-917.
19. Kothary, M.H., and Kreger, A.S. (1985) *Infect. Immun. 49,* 25-31.
20. Hong, C.I., An, S., Buchheit, D.J., Nechaev, A., Kirisits, A.J., and West, C.R. (1986) *J. Med. Chem. 29,* 2083-2044.

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